INFLUENCE OF DATE OF HARVEST ON YIELDS AND LEAF CARBOHYDRATES OF CITRUS

By

Juan Manuel Ramirez

A Dissertation Presented to the Graduate Council of
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To my daughter Erika

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June, 1977

Chairman: Dr. A. H. Krezdorn
Major Department: Horticultural Science

Holding crops of 'Hamlin' and 'Valencia' oranges and 'Marsh' grapefruit on the tree until late in the season depressed yields the following year. Yields of 'Valencia', a late-maturing cultivar, were reduced the most. Yields of 'Hamlin', an early-maturing cultivar, were reduced in both years by relatively heavy fruit drop late in the season and by the fact that fruit did not increase in size throughout the time it was held on the tree, as was true for 'Marsh' and 'Valencia'.

Samples of leaves were analyzed periodically for carbohydrates to determine their relation to flowering and fruiting. Levels of carbohydrates in the leaves of all cultivars were depressed just prior to the bloom by late dates of harvest; however, the following bloom and growth were not related to these carbohydrate levels.

Time of fruit harvest did not influence the levels of carbohydrates in new leaves of spring growth the following year. Thus, carbohydrate levels did not appear to limit flowering and fruiting.

Chairman

INTRODUCTION

Alternate bearing is a term used to describe the tendency of many tree fruits to produce a large crop one year and a smaller one the next. Some citrus cultivars exhibit a pronounced alternate-bearing cycle, while others are less affected.

The best-documented condition leading to alternate bearing in citrus is holding fruit on the tree long past the attainment of legal maturity (4, 20, 34, 38, 43, 46, 50, 73). This condition has been studied most thoroughly in California, where 'Valencia' orange displays much more severe alternate bearing than in Florida. This is probably due in California to the later date when legal maturity is attained as a result of cooler climatic conditions during fruit development, most of which takes place in the fall and winter months. This necessitates fruit being held on the tree there as late as Oct. of the year following that in which it bloomed. The 'Valencia' season is complete in Florida by June or July.

Research in California indicates that late storage of fruit on the tree depleted carbohydrate (CHO) reserves, thereby reducing the amount of bloom, yield

and fruit size (38, 45). More recent work in California (47, 51, 56) suggests CHO's may not be the only controlling factor in the biochemistry of fruiting; however, the evidence still shows CHO's are important in the fruiting process.

The only study of CHO's in leaves of citrus in Florida is that of Smith, Reuther and Specht (84), who reported the seasonal changes in mineral elements and CHO's in 'Valencia' orange leaves. They did not attempt to relate their results to yields or flowering processes.

Alternate bearing is not as serious a problem in Florida as in California, but it is a matter of concern. Moreover, there is an increasing tendency to hold fruit on the tree later than in the past in order to extend the harvest season. Even fruits of 'Hamlin' orange, an early-maturing cultivar, are being held on the tree long past their date of first legal maturity in order to develop higher concentrations of sugars in the juice and thereby make them more valuable for processing. Thus, the objectives of this research were to determine the influence of time of harvest on the yield and CHO content of 3 citrus cultivars and whether CHO levels were related to flowering and fruiting or neither of them.

LITERATURE REVIEW

Introduction

Alternate bearing, *biennial* bearing and *erratic* bearing are terms used to denote the characteristic of many fruit cultivars to bear extremely large crops in certain years and much smaller ones in others. Pronounced heavy and light crops occur in some cases, but in others the differences in yield are not as constant. Apple (26, 27), avocado (37), pecan (88) and pistachio (15, 16) are examples of fruits which show pronounced alternate bearing. Peach (10) and fig (10), on the other hand, are examples of fruit crops that rarely if ever exhibit this tendency. The jaboticaba (69), on the other hand, flowers and matures fruits about every 2 months when growing conditions are satisfactory. Citrus cultivars vary considerably in their tendency to alternate bearing, but it is a major problem (3, 4, 20, 34, 38, 45, 46, 47, 50, 62, 63).

Factors Influencing Alternate Bearing

Species and Cultivar

Some mandarin (*Citrus reticulata* Blanco) cultivars,

such as 'Dancy' and 'Kinnow', are pronounced alternate bearers (21, 32, 33, 48), while others, such as satsuma (*C. unshiu* Markovitch), fruit regularly (94). The 'Minneola' tangelo (*C. paradisi* x *C. reticulata*) has a similar tendency, while 'Orlando' tangelo (*C. paradisi* x *C. reticulata*), a sibling cultivar, fruits regularly. Lemons (*C. limon* (L.) Burm. f), lime (*C. aurantifolia* (Christm.) Swing.) and citron (*C. medica* L.) not only fruit regularly but produce some flowers and fruit throughout the year. Similarly, calamondin (*C. madurensis* Lour.) fruits all year. Seedless grapefruit (*C. paradisi* Macf.) shows a moderate tendency to alternate bearing; however, seedy cultivars may alternate badly. 'Pineapple' and 'Valencia' sweet oranges (*C. sinensis* (L.) Osbeck) may alternate strongly, while 'Hamlin' is a consistent bearer. Alternate bearing of seedy cultivars is much more pronounced when minor elements are deficient, but some persists even under the best nutritional program.

Climate

The various species of citrus and even horticultural cultivars vary in their reaction to climatic conditions. External appearance and juice quality are greatly

influenced by climate (74, 75, 76), and some species are much more cold hardy than others.

The influence of climate on alternate bearing is indirect. For example, 'Valencia' orange, which often bears alternately, requires 9 to 10 months to ripen in Colombia, 10 to 11 months in Florida and up to 15 months in California, the differences in months from bloom to maturity being directly related to temperatures during the fruit development period (75, 76). 'Valencia' fruit must remain on the tree long after bloom in California, and there are 2 crops on the tree for as much as 6 months. It is noteworthy that those areas of the world, such as Australia (20, 62, 63) and South Africa (4), in which 'Valencia' suffers most severely from alternate bearing are those with climates similar to California. 'Valencia' is not only a regular bearer but may set and mature 2 crops annually in the hot tropics.

The nature of the dormant period also plays a role. Vegetative growth and bloom are regulated by temperature in subtropical areas, with trees exhibiting a distinct winter dormant period. A heavy, single, concentrated spring bloom follows the winter dormant period. Dormancy and growth in the hot tropics, on the other hand, is regulated by rainfall, and the bloom is less distinct

and generally lighter than in the subtropics (74). Some cultivars set excessive crops in subtropical regions as a result of heavier bloom. This accentuates alternate bearing (74, 76).

Cultural Practices

Fruit thinning, the removal of a portion of a crop well before fruit maturity, is commonly used in some species to reduce the demand of water and photosynthates and thereby increase fruit size (1, 9, 10). Thinning fruits is also thought to contribute to more regular bearing (26, 27), heavy thinning being done in the "on" year. This has been shown to decrease the current yield on a volume basis and to increase the amount of bloom the following year (10, 27, 32, 33, 67).

Fruit thinning is not a common practice in citrus. Gardner et al. (21) reported that thinning 'Dancy' tangerines in Florida reduced total yield but there were as many large marketable fruits on the unthinned as on the thinned trees, and he did not relate this to fruiting the next year. Early removal of a part of the crop of 'Wilking' and 'Kinnow' mandarins (in California) in the "on" year, however, did result in less reduction in yield the following year (32, 33). Hilgeman et al. (36) found

that removal of over 50% of the fruit during an "on" year (in Arizona) was needed to eliminate alternate bearing.

Pruning has been used in a number of instances to modify alternate bearing through reduction of the crop and stimulation of vegetative vigor. Moderate pruning of apples during the "on" year has reportedly reduced alternate bearing slightly, but it did not overcome the problem (27). Extremely heavy pruning of apples was more effective through its reduction of the crop, but the reduction of yield was excessive (24, 26). Moderate pruning of pecans reportedly stimulated growth and prolonged retention of leaves in the fall. This resulted in a reduced tendency to alternate bearing (82).

It has been suggested that certain mandarin cultivars which set excessively heavy crops and suffer from severe alternate bearing should be pruned heavily to reduce the crop and stimulate vegetative growth (8). There has been little research to investigate this possibility; however, skeletonizing 'Mediterranean' mandarin (removal of the entire canopy by cutting back to framework limbs 2.5 to 5 cm in diameter) reportedly eliminated alternate bearing through 3 2-year cycles (71). Inspection of the data, however, shows that the tendency to alternate

bearing returned as the yields of pruned trees approached those from unpruned plots. Total yields from the pruned and unpruned trees respectively were, however, about the same when both "on" and "off" years were considered. Florida growers commonly cut back the tops and sides of 'Dancy' tangerine trees annually to reduce the crop and produce vigorous new wood. Growers feel that this results in larger fruit and more regular bearing, even though total yield is reduced. This has not been substantiated through research, but the continuation of the practice suggests it is effective.

The large influence of N on yields through its influence on fruit set has resulted in the manipulation of applications of this mineral element to regulate yields. Primarily, N levels have been reduced prior to flowering in the "on" year to reduce fruit set, with an application after fruit set to maintain vegetative vigor (24). At best, fertilizer practices mitigate rather than eliminate alternate bearing. Late summer applications of N fertilizer to apples resulted in late retention of leaves (30). This resulted in both an increase in the rate of production of CHO's and continued production after leaves of trees not receiving N in the late summer had ceased production. High N

treatments also produced a larger leaf surface the next spring. Alternate bearing was reduced but not eliminated.

Practices which maintain an adequate leaf area by protecting them from disease and insect damage have also been effective in reducing alternate bearing. There is a substantial quantity of data for pecans showing that leaf diseases and early defoliation in the fall result in reduced quality of the fall-maturing crop and a failure to form flowers the next spring (30, 47, 57, 80, 81, 82, 87, 93). Greasy spot (*Mycosphaerella citri* Whiteside) fungus may cause excessive leaf drop in citrus and greatly reduce yields (41), thus accentuating a tendency to alternate bearing. Numerous other diseases, insects and mites may reduce the effectiveness of citrus leaves, but research has not measured their influence on yields or the ability of the leaf to function at maximum effectiveness. The consistency of the relation of crop load and leaf to fruit ratio to flowering and alternate bearing suggests, however, that any factor which causes a reduction in effectiveness of the leaf in producing photosynthates may influence flowering and alternate bearing (9, 10, 24).

Time of Harvest

Crop load and time of fruit removal have been found closely associated with alternate bearing of

citrus in several areas of the world. In an 8-year study of differential harvesting of 'Valencia' orange in California (38), some trees were completely harvested in Feb., Apr., June, Aug. and Sept. Other treatments consisted of removing half of the crop at those intervals, with the remaining fruit harvested on Oct. 15. Both harvesting early and partial harvesting reduced the normally depressing effects of the "on"-year crop. Fruit size was not related to amount of crop. 'Valencia' oranges were harvested early (June 4), midseason (July 24) and late (Sept. 21) in another long-term experiment (46). Delaying harvesting decreased yields and increased the tendency to alternate bearing (43, 46). It was concluded from yet another experiment in California (50), which lasted 14 years, that harvesting well after the attainment of legal maturity results in reduced fruit yield and lower grade and that a late harvest increases alternate bearing. Studies with 'Valencia' orange in Arizona (34), in which fruit was harvested in late Feb. each year; late May each year; alternating Feb., then May; and alternating May, then Feb., demonstrated that trees harvested in Feb. produced more fruit per tree than those harvested in May in 6 out of 8 years. Reversing the

time of harvest reversed the alternate bearing the first subsequent year.

Research in South Africa (4) showed that the later 'Valencia' fruit were harvested, the lower were the yields of the ensuing crop. Picking after full bloom was directly related to a decrease in fruit weight and number of fruit per tree; however, a direct effect on fruit set was not observed. A study on the effect of time of picking of 'Valencia' orange in India at monthly intervals from Feb. to May showed that depression in yield was becoming apparent after 2 years of consistently delayed harvest, up to Apr. and May. Harvesting the heavy crop early and the small crop late alleviated the alternate bearing of 'Valencia' orange to some extent in Australia (20).

Heavy thinning combined with early harvest of satsuma in Japan (67) favored flower formation the following season. Delayed harvest reduced the stored food supply for the next crop. Early harvest increased the number of flowers which developed the following year but did not affect the number of shoots produced in other work with satsuma (42). Fruit thinning combined with moderate pruning and abundant fertilization stimulated flowering and fruit production of satsuma the next year (68).

The only research with late harvest in Florida has been with 'Marsh' grapefruit (73) where the fruit was harvested in Oct., Dec., Feb. and Apr., respectively. Part of the trees were partially harvested (spot picked) in Oct. while others carried the full crop until harvest. Late harvesting reduced yields the following year, when fruits from all plots were harvested in Dec. Spot picking materially reduced the depressing effect of holding fruit on the tree late.

Physiology of Alternate Bearing

CHO's constitute the major energy supply of most plants, with starch the principal reserve form. It is well established that seasonal changes in levels of reserve and available CHO levels are related to both vegetative growth and the fruiting process. Cameron (6) determined the seasonal distribution of starch in all parts of the 'Valencia' orange tree in 1932. The level of starch was at a minimum in July and Aug. and increased throughout the remainder of the spring and summer. The fact that there was a significant increase in starch content in leaves during the autumn and winter suggests that they were functioning as storage organs, representing as they do approximately 25% of

the tree's fresh weight. Cameron and Schroeder (7) found that initiation of cambial activity in the spring was basipetal in both shoots and roots. Fluctuations in starch as determined by an iodine test were confined to tissues close to the cambium and to parts adjacent to actively growing shoots and roots. Other parts of the plant contained large unfluctuating quantities of starch. Heavy fruiting was followed by a reduction in starch, mainly in twigs and small branches. Leaves were not analyzed. These data suggest that the amount of locally stored or produced CHO's plays a major role.

There have been several studies of seasonal changes of CHO's in citrus leaves. Both starch and sugars in 'Valencia' orange (6, 35, 49) were at a minimum during the summer in California. Starch remained low during the winter, but sugars were then at a maximum. Starch, however, increased rapidly to a maximum just prior to flowering and growth in early spring. Similar results were obtained with navel oranges (18). A study of seasonal changes of 'Marsh' grapefruit in Arizona (79) showed considerable accumulation of starch in winter months, with a maximum reached in early spring. Starch decreased to an extremely low level during the main season of vegetative growth in the summer. Starch

reportedly decreased in mandarin leaves in studies in India from Oct. to Jan. However, it increased in Feb. just prior to bloom (52).

There has been a dearth of work with CHO's in Florida, the only work being that of Smith, Reuther and Specht (84), who reported starch was present in low amounts in 'Valencia' orange leaves throughout the year. Winter and early spring accumulations were exhausted soon after new growth began. Total sugars varied widely from month to month, but the greatest losses occurred during spring growth and bloom. Thus, the seasonal changes in CHO's of sweet orange, grapefruit and mandarin follow essentially the same pattern. Lemon, however, differs in that starch continues to increase well past the bloom period (18).

Presence of fruit on the tree is considered as an increased demand on CHO's produced by leaves due to respiratory losses and increases in fruit size and sugar components. It is also well accepted that CHO's comprise the major material used in growth and development of all plant parts. Some research has been conducted to determine whether depletion of CHO's resulting from such factors as heavy crop load or late harvest is related to alternate bearing.

Virtually all of the research relating to the relationship of CHO's to alternate bearing has been done in California and Arizona, where this is a severe problem. Jones and various coworkers (45, 46, 47, 50) reported delaying harvest of 'Valencia' orange in California resulted in lower yields the following year, which they related to a reduction of leaf CHO's just prior to bloom in the spring. They suggested that this reduction in CHO's constituted the major factor limiting fruiting. Hilgeman, Dunlap and Sharples (35) in Arizona corroborated the California results. Later, Jones et al. (51) reported that sufficient flowers were produced on 'Valencia' trees despite lower leaf CHO's resulting from delayed harvest. Thus, they postulated lower yields resulting from late-held fruit were due to competition between old fruit and new fruit for photosynthates produced by new leaves. Fruit-thinning experiments in recent years, however, have led to the conclusion that CHO's may not be the limiting factor controlling fruiting (47). Lewis, Coggins and Hield (56) changed the alternate-bearing cycle of 'Kinnow' mandarin by thinning the crop with α-naphthaleneacetic acid (NAA). Leaf CHO content was not significantly affected. They proposed an NAA-sensitive mechanism.

Jones et al. (47) thinned 'Valencia' oranges in 1970 (an "on" year) 0, 33, 66 and 100%. The amount of fall growth was increased by fruit removal, and the spring flush was earlier and more extensive when 100% of the fruit was removed. There was a positive correlation between fruit set in the spring of 1971 and starch content in leaves before the beginning of spring growth. There was an increase in starch, but not in sugars, but these differences had disappeared by the end of the June drop. They concluded that CHO's did not limit fruit set. These results and others of similar nature with 'Wilking' and 'Kinnow' mandarins (11, 32, 33, 36, 56) supported the conclusion that CHO's were not the limiting factor involved. It was postulated that endogenous growth regulators control flowering (51).

There is evidence that growth regulators or hormones are involved. External applications of gibberellins (GA) have reduced flowering of citrus (12, 13, 14), and gibberellins have been identified in citrus (23, 54, 91). One hypothesis is that a gibberellin-like substance might diffuse from the fruit tissue into the shoot on which it is borne, since practically no flowers were produced on shoots with fruit (62). Applications of a nucleic acid

(5-fluorodeoxyuridine plus thymidine) during the flower induction period increased bud sprouts, laterals per branch and flowering. This might be related to the repression of synthesis of a proteinaceous flower inhibitor which might act directly or through hormonal balance (44). There is some evidence, however, that growth regulators simply act to mobilize and control CHO's. Haas (25), Moss (60) and Lenz (55) showed that sweet oranges did not set appreciable fruit until the latter part of the bloom, which they related to the maturing of spring growth leaves and their change from parasitic to productive organs. Moss (61) could find no evidence of chemical regulation of fruit set associated with these leaves. Moss, Steer and Kriedemann (64) used ^{14}C-labeled photosynthates and radioautography to show that these leaves from flowering shoots were major sources of CHO's for fruit set. Powell (70), using similar techniques, showed that flowers of leafless shoots could be induced to set fruit with GA applied to flowers. Gibberellic acid apparently caused a movement of ^{14}C-labeled photosynthates from old leaves to young fruit. Export was basipetal in controls. Leaves from flowering shoots, on the other hand, exported photosynthates to young fruit without the application of GA as soon as they were expanded to two-thirds full size.

Methods of Determining Carbohydrates

Numerous methods have been used to determine levels of CHO's in citrus since the first analyses were made in 1932 (6). The methods vary considerably, and no method has become standard.

The picric acid colorimetric method (Benedict-Lewis method) was modified by Willaman and Davison in 1924 (90). Modifications included the color standard, heating procedure, dilution of the unknown, clarification, calculation of results and color factors developed for the various reducing sugars. Much practice is needed for obtaining comparable results.

One of the most accurate and convenient methods for the determination of reducing sugars in solution (after clarification) is the use of copper reagents of the type involving iodometric titration of cuprous oxide. Analytical errors will depend chiefly upon the composition of the copper reagent and the conditions which affects its sensitivity and reproducibility during sugar oxidation. Shaffer and Somogyi (78) improved these methods in 1933 by studying the various factors influencing the rate of copper reduction and the maximum reduction equivalent per unit of sugar in order that the method could be applied to sugars other than glucose or to mixtures of sugars. They found several reagents are needed since none is optimum for all sugars.

Hassid (28) devised a new method in 1936 as a substitute for the Munson and Walker method. Reducing sugars in the clarified extract are oxidized with alkaline potassium ferricyanide. Ferrocyanide formed in the reaction is titrated in acid solution with ceric sulfate, which oxidizes the ferrocyanide back to ferricyanide to give a measure of the reducing sugars present.

Forsee (19) modified Hoffman's method (39) in 1938 for citrus sugars. He made use of the fact that ferricyanide solutions are colorless. Glucose in the clarified extract is estimated by measuring the diminution in yellow color of an excess of ferricyanide in a colorimeter. It gave a good recovery of glucose and sucrose added to the plant material.

Hassid et al. (29) proposed hydrolysis of starch by salivary amylase in 1940, since it shortened the procedure. The hydrolyzed solution was clarified, deleaded with sodium phosphate, and the maltose produced from the hydrolyzed starch was determined with his method above.

Heinze and Murneek (31) compared 5 of the most common methods of CHO analyses in 1940. Bertrand's method was found to be the most accurate (5), but that of Shaffer-Somogyi was almost as accurate and simpler.

Nielsen (66) found in 1943 that the time required for "solubilization" of starch could be shortened with

perchloric acid. Starch was then estimated by Pucher and Vickery's starch-iodine clorimetric method (72).

Shaffer-Somogyi (78) and Somogyi's (85) methods are quite reliable for sugar determination. They can be adapted to colorimetric use by omitting the iodide and iodate in their preparation, since these compounds interfere with the molybdenum color reagent and their omission produces no special change in the character of the reagents. Potassium iodide inhibits the autoreduction of the copper, so its absence causes the reagent to be unstable. This difficulty is avoided by adding copper the day of use. The color is unstable when the Somogyi micro-reagent (85) is used in this way with almost any of the various phosphomolybdate reagents. Nelson (65) tried to avoid the above problem with various color reagents in 1944. This led to the development of the arsenomolybdate color reagent, which made it possible to utilize the copper reagents in a photometric procedure for practically all uses for which the titrimetric procedures are adapted. The chemistry involved in the molybdenum blue reaction is unknown (92), but there is some indication that the formula of the blue material separated may be expressed as $H_3AsO_4(4MoO_3.MoO_2)_2$.

Scott and Melvin (77) studied and improved the reaction obtained by heating CHO's with anthrone in sulfuric acid in 1953 to determine the concentration of dextran solution. The method is very precise if blanks are carefully prepared. However, Dugger and Palmer (18) pointed out that acid hydrolysis of the extract hydrolyzes sugar molecules from flavonoid molecules, thereby adding them to the pool of free sugars.

Keston (53) and Teller (89) developed an enzymatic determination of glucose in 1956. The method is based on a specific oxidation of glucose by glucose oxidase (reaction 1) and the determination of the resulting peroxidase in the presence of a suitable chromogenic oxygen acceptor (reaction 2)

$$\beta\text{-D-glucose} + O_2 + H_2O \xrightarrow{\text{glucose oxidase}} H_2O_2 + \text{D-glucone-}\delta\text{-lactone} \quad (1)$$

$$H_2O_2 + \text{reduced chromogen} \xrightarrow{\text{peroxidase}} \text{oxidized chromogen} + H_2O \quad (2)$$

Color produced is proportional to the amount of glucose present. Hugget and Nixon (40) adapted these reactions for the determination of blood glucose. Enzymatic digestion of the starch has been mostly done with takadiastase

(45, 46, 47, 49, 50, 51) or with amyloglucosidase (15, 17, 58, 59). A digestion period of 12 hr is required for the takadiastase and only 1.5 hr for amyloglucosidase. Then it is assayed by any of the methods for glucose determination and reported as glucose equivalent.

Sucrose has been determined by hydrolyzing it with invertase (18, 56). Glucose and fructose from sucrose are added to the existing pool of reducing sugars to give total sugars, and then reducing sugars are determined by any of the methods given above. The difference between "total sugars" and reducing sugars analyzed previously gives the sucrose content as glucose equivalent.

Thus, the methodology of CHO analyses varies considerably, and no method has become standard. The time-consuming nature of CHO analyses, while still present, has been greatly reduced, so that studies requiring them are now more practicable.

MATERIALS AND METHODS

Experiments were conducted with 22-year-old 'Marsh' grapefruit, 22-year-old 'Hamlin' and 8-year-old 'Valencia' orange trees, respectively, all on rough lemon rootstock. Trees were grown on an Astatula fine sand in Lake County under standard commercial programs of fertilization and pest control. The 'Marsh' trees were irrigated and also given a post-bloom lead arsenate spray to prevent development of high fruit acidity. 'Marsh' and 'Hamlin' were hedged in alternate rows in Apr. and May 1974, respectively, after fruit was harvested. 'Valencia' trees were not large enough to require hedging.

A randomized complete block design with single-tree plots was used. There were 28, 21 and 50 replications in the 'Marsh', 'Hamlin' and 'Valencia' experiments, respectively. There were 4 dates of harvest, which constituted treatments, in each experiment (Table 1).

Fruit Size, Quality and Yield

Fifty random-sized fruit were taken from around each tree from 1.5 to 2.0 m above ground level at the

time of harvest. Alternate fruit were harvested from the outer and inner leaf canopies.

Total soluble solids (TSS), total titratible acid (TTA) and percent of juice on a weight basis (percent juice) were determined for each sample according to standard methods (86).

Yield was measured both as number of field boxes (38.6 kg for grapefruit and 40.8 kg for oranges) per tree and kg of soluble juice solids (kg solids) per tree (86).

Carbohydrate Analyses

Forty shoots from the spring flush were selected from around the outer canopy of each tree from 1.5 to 2.0 m above the ground and tagged. 'Hamlin' was sampled between 9:30 AM and 11:30 AM, 'Marsh' between 9:30 AM and 12:30 PM and 'Valencia' between 9:30 AM and 1:00 PM, when seasonal changes were studied. A longer time was required to obtain leaves when all treatments for a given cultivar were taken because almost 4 times as many trees were involved. This sampling was done in a single day between 9:30 AM and 2:30 PM for 'Hamlin' and 'Marsh'. Half of the 'Valencia' trees were sampled between 9:30 AM and 2:00 PM one day, and the other half between the same hr the following day. The long period

of time required for sampling was not desirable, because CHO's may change during the day (Figs. A, B and C of the Appendix). This error was compensated for by sequential sampling of the blocks in each experiment. Thus, differences between blocks were, in part, differences in time. Variability due to time was removed statistically. A leaf was taken from each shoot at dates indicated in the schedule (Table 1).

Leaves of each sample were put immediately upon removal into a plastic bag which was then immersed in a cold bath of dry ice and acetone (-56.7°C) for 3 to 5 min. The bags were placed in a chest with dry ice and transported to Gainesville. Fresh weights of leaves were obtained, after which leaves were dried in a forced draft oven at 60°C for 12 hr, ground in a Wiley mill, passed through a 40-mesh screen and stored in closed glass bottles. They were ground again, using a 60-mesh screen, just prior to analyses.

A diagram of the analytical procedure is given in Fig. 1. Results are reported as glucose equivalent on a percent dry weight basis for total CHO (total sugars plus starch), total sugars (reducing sugars plus sucrose) and starch.

Table 1. Schedule for harvesting fruit and sampling leaves.

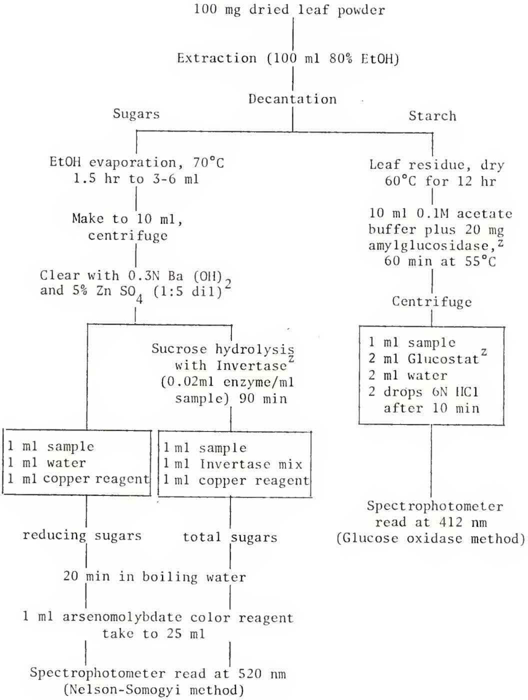
Treatments sampled	Cultivar		
	Marsh	Hamlin	Valencia
	1974-75^{z}		
All treatments	Oct. 29	Nov. 24	Apr. 17
2nd harvest date	Dec. 30	Dec. 28	May 15
3rd harvest date	Feb. 17	Jan. 30	June 12
4th harvest date	Apr. 12	Feb. 25	July 18
	1975-76^{y}		
All treatments	Oct. 21	Nov. 18	Apr. 14
2nd harvest date	Dec. 30	Dec. 29	May 21
3rd harvest date	Feb. 24	Jan. 20	June 18
4th harvest date	Apr. 30	Feb. 15	July 12
Just before bloom	Feb. 7	Feb. 15	Feb. 14
All treatments	May 7^{x}	May 12^{x}	July 16^{x}

^{z}Mature leaves from spring flush 1974.

^{y}Mature leaves from spring flush 1975, except May 7 and 12 and July 16 sample dates.

^{x}Young leaves from spring flush 1976.

Fig. 1. Diagram of analytical procedures for carbohydrates.



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[z]Amyloglucosidase from Sigma Chemical Co.; Invertase concentrate in glycerol from B.D.H. Laboratories, London, England; Glucostat and chromogen from Worthington Biochemical Co.

Estimates of Bloom and Vegetative Growth

Flowering of 'Marsh' and 'Hamlin' trees was evaluated Feb. 25 in both 1975 and 1976, and that of 'Valencia', on March 1, 1976 according to the scale of 1 = nil or sparsely scattered, 2 = bloom over half of the canopy or 3 = canopy fully covered with bloom.

The scale used for the amount of new growth, which was evaluated for all cultivars on Mar. 30 in 1975 and 1976 for 'Marsh' and 'Hamlin' and only in 1976 for 'Valencia', was 1 = nil or a few scattered new shoots, 2 = half-covered with new growth or 3 = new growth over the entire canopy.

RESULTS AND DISCUSSION

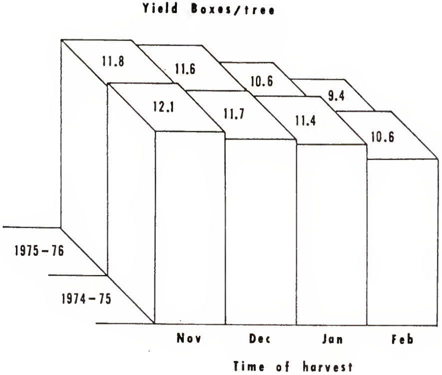
Yield

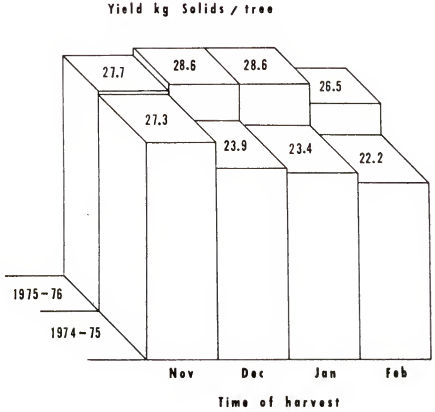
'Hamlin' Orange

Yields of 'Hamlin' on a volume (box) basis (Fig. 2) decreased in both years with each later date of harvest. Mean differences were not always statistically significant, but yield of trees harvested at any given date was always less than that of yields of any previous date the same season. Moreover, yields of trees harvested the latest date of a season were always significantly lower from at least the 2 earliest dates of harvest. Differences between the first and last dates of harvest were 1.5 and 2.4 boxes for the 1974-75 and 1975-76 seasons, respectively. These quantities are economically important.

The pattern of yields is different from those previously reported for 'Marsh' grapefruit (73) and 'Valencia' orange (34, 35, 50), where the yields increased with each successively later date of harvest the first season. Decreases in yields of late-harvested 'Hamlin' the first year were due in large part to fruit drop and to the fact that 'Hamlin' fruit does not

Fig. 2. Effect of date of harvest on yield in boxes and kg solids per tree of 'Hamlin' orange.


Yield Boxes/tree
11.8
11.6
10.6
9.4
12.1
11.7
11.4
10.6
1975-76
1974-75
Nov
Dec
Jan
Feb
Time of harvest


Yield kg Solids / tree
27.7
28.6
28.6
26.5
27.3
23.9
23.4
22.2
1975-76
1974-75
Nov
Dec
Jan
Feb
Time of harvest

continue to increase in size throughout the season. No data were obtained on fruit drop, but much larger quantities of fruits were observed under trees harvested at successively later date. Fruit size increased slightly between the Nov. and Dec. harvests but stayed the same for the last 3 dates in both seasons (Table 2). The influence of late harvest on yields the following year was not as pronounced as has previously been reported for late-maturing cultivars (34, 38, 43, 46, 50). This was not surprising, because the latest harvest date for 'Hamlin' was prior to bloom and much over a month earlier than the first 'Valencia' harvest. Even so, differences between yields of the earliest- and latest-harvested trees were greater for the second season than for the first, indicating late harvesting of 'Hamlin' did depress yields slightly the following year.

A large part of the 'Hamlin' crop is sold for juice on a kg solids basis. These yields gave a somewhat different pattern because of variations in percent juice and TSS. It is generally assumed that TSS and percent juice will increase during the first part of the season and possibly decline as fruit becomes senescent. Total soluble solids did increase during the first season, but

Table 2. Effect of date of harvest on yield,[z] quality,[y] fruit size,[x] amount of bloom[w] and new growth[v] of 'Hamlin' orange.[u]

Harvest date	Yield/tree	Solids/tree	Juice	TSS	TTA	Fruit diam	Bloom rating	New growth rating
	(boxes)[t]	(kg)	(% by wt)	(%)	(%)	(cm)		
				1974-75				
Nov. 24	12.1a	27.3a	58.8a	9.4b	0.78a	7.3b	3.0a	3.0a
Dec. 28	11.7a	23.9b	52.3b	9.6ab	0.75b	7.4a	3.0a	2.9a
Jan. 30	11.4ab	23.4b	51.3b	9.8a	0.64c	7.4a	2.9a	3.0a
Feb. 25	20.6b	22.2b	51.6b	9.9a	0.60d	7.4a	2.9a	2.9a
				1975-76				
Nov. 18	11.8a	27.7a	54.7a	10.5d	0.89a	6.7b	3.0a	3.0a
Dec. 29	11.6a	28.6a	53.2a	11.4c	0.83b	6.8a	2.9a	3.0a
Jan. 20	10.6b	28.6a	55.5a	12.1b	0.80c	6.8a	2.8a	3.0a
Feb. 23	9.4c	26.5a	55.2a	12.8a	0.67d	6.8a	2.8a	2.8b

[z]Average based on 21 trees.

[y]Random sample of 25 fruit.

[x]Random sample of 50 fruit.

[w]1 = nil or sparse and scattered; 2 = bloom over half of canopy; 3 = canopy fully covered with bloom.

[v]1 = nil or few scattered shoots; 2 = half-covered with new growth; 3 = new growth over entire canopy.

[u]Means in each column followed by the same letter do not differ statistically at the 0.01 level.

[t]40.8 kg standard box.

[s]Solids/tree = (boxes x wt) x % juice x $\frac{\%\ TSS}{100}$

percent juice decreased (Table 2). The increase in TSS was not enough to overcome the loss in boxes of fruit.

Total soluble solids increased the second year as the season progressed while percent juice remained essentially the same. Yields were not statistically different even though there was a mean difference of 2.1 kg solids between the Jan. and Feb. harvests. It is possible, of course, that there would be some seasons in which a higher or lower yield would result from date of harvest, but it appears that differences will be less pronounced than for yields on a volume basis.

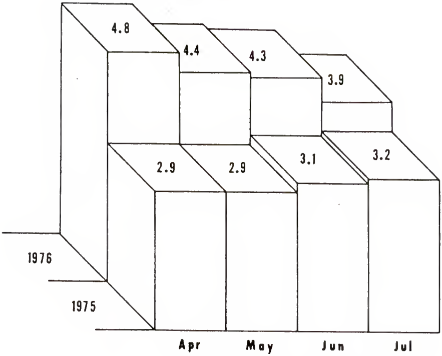
The advantage of prolonging the harvest season must be balanced against the lower yield resulting from late harvest and the possibility of losses from freezes, as was experienced in 1977.

'Valencia' Orange

'Valencia' orange, unlike 'Hamlin', increased in yield with successively later dates of harvest during the first season (Fig. 3). These increases were primarily due to increases in fruit diam (Table 3). Differences were not statistically different even though there was about a 10% mean difference between the first and last dates of harvest. The stepwise

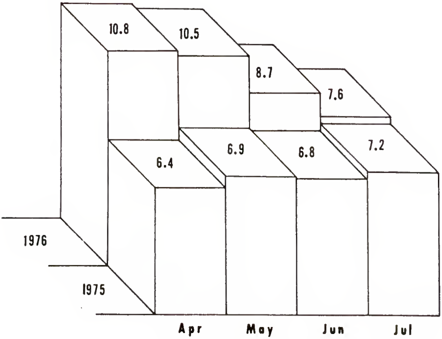
Fig. 3. Effect of date of harvest on yield in boxes and kg solids per tree of 'Valencia' orange.

Yield Boxes/tree



Time of harvest

Yield kg Solids / tree



Time of harvest

Table 3. Effect of date of harvest on yield,[z] quality,[y] fruit size,[x] amount of bloom[w] and new growth[v] of 'Valencia' orange.[u]

Date of harvest	Yield/tree	Solids/tree[s]	Juice	TSS	TTA	Fruit diam	Bloom rating	New growth rating
	(boxes)[t]	(kg)	(% by wt)	(%)	(%)	(cm)		
				1975				
Apr. 17	2.9a	6.4a	48.2b	11.2b	1.00a	7.1c	-	-
May 15	2.9a	6.9a	48.6b	11.7a	0.98b	7.1c	-	-
June 12	3.1a	6.8a	49.1b	11.0b	0.75c	7.4b	-	-
July 18	3.2a	7.2a	52.9a	10.1c	0.63d	7.5a	-	-
				1976				
Apr. 14	4.8a	10.8a	50.6a	10.9b	0.92a	7.2c	3.0a	3.0a
May 21	4.4ab	10.5a	51.3a	11.7a	0.78b	7.2c	3.0a	3.0a
June 18	4.3ab	8.7b	47.9b	10.2b	0.63c	7.6b	2.9a	2.9a
July 12	3.9b	7.6b	47.7b	9.9c	0.59d	7.7a	2.9a	3.0a

[z]Average based on 50 trees.

[y]Random sample of 25 fruit.

[x]Random sample of 50 fruit.

[w]1 = nil or sparsely scattered; 2 = bloom over half of canopy; 3 = canopy fully covered with bloom.

[v]1 = nil or few scattered shoots; 2 = half-covered with new growth; 3 = new growth over entire canopy.

[u]Means in each column followed by the same letter do not differ statistically at the 0.01 level.

[t]40.8 kg standard box.

[s]Solids/tree = (boxes x wt) x % juice x $\frac{\%\ TSS}{100}$

increases, however, suggest the differences were real. Harvest dates the first year influenced the yields the second year in that the pattern of yields was reversed, the trees harvested in Apr. having significantly more fruit than those harvested in July. This influence is well documented by research in other regions (34, 35, 46, 47, 50). No other research, however, has reported yields on the basis of kg solids.

Patterns of yields, unlike those for 'Hamlin', were essentially the same on a volume and kg solids basis. The pattern of TSS was the same both seasons; whereas percent juice remained the same for the first 3 harvest dates and increased for the last date in 1975. The percent juice remained the same for the first 2 harvest dates, but it was lower for the last 2 harvest dates in 1976. These differences in juice quality either offset each other or were too small to change the pattern of yields as kg solids from that on a volume basis. Quality of juice varies considerably from year to year, and it is very likely that the pattern could be changed to a limited degree; however, the pronounced influence of date of harvest on yield on a volume basis suggests that this will be the controlling factor.

'Marsh' Grapefruit

'Marsh' grapefruit, like 'Hamlin' orange, reaches legal maturity in the fall, but like 'Valencia' it may be retained on the tree until summer, during which time it continues to increase in size. Yields of 'Marsh' grapefruit increased with late dates of harvest during the first season (Fig. 4). Differences were not statistically significant, however, even though mean difference in yield between the Oct. and Apr. harvest dates was 1.4 boxes. The largest difference between 2 successive harvest dates was 0.9 box between Oct. and Dec. The progressive increases in yield with successively later harvest dates were due to increases in fruit size as the season progressed (Table 4). The agreement of these data with those of previous work in the same planting (73) and with work in other regions suggests the differences were real despite lack of statistical significance. Yields during the second season increased through the Feb. harvest by a total of 0.9 box, but holding fruit until Apr. resulted in a decrease of 0.5 box from the Feb. harvest (Fig. 4). Neither of these differences were significant.

Yields the first season were all lower than those of the second season, indicating treatments of various

Table 4. Effect of date of harvest on yield,[z] quality,[y] fruit size,[x] amount of bloom[w] and new growth[v] of 'Marsh' grapefruit.

Date of harvest	Yield/tree	Solids/tree[s]	Juice	TSS	TTA	Fruit diam	Bloom rating	New growth rating
	(boxes)[t]	(kg)	(% by wt)	(%)	(%)	(cm)		
			1974-75					
Oct. 29	13.4a	21.1a	48.2	8.3a	0.95a	10.5b	3.0a	2.9a
Dec. 30	14.3a	20.6a	46.6ab	8.4a	9.92a	10.7b	3.0a	2.9a
Feb. 17	14.5a	20.9a	45.0b	7.9b	0.84b	10.9ab	3.0a	2.7a
Apr. 12	14.8a	19.6a	46.6ab	7.7c	0.66c	11.0a	3.0a	2.8a
			1975-76					
Oct. 21	16.2a	24.3b	47.2b	8.2a	1.02a	9.4d	2.9a	3.0a
Dec. 30	16.7a	28.8a	51.8a	8.5a	0.93b	10.1c	3.0a	3.0a
Feb. 24	17.1a	28.2ab	51.4a	8.2a	0.85c	10.3b	2.9a	2.9a
Apr. 30	16.6a	23.8b	47.9b	7.9a	0.66d	10.5a	2.9a	3.0a

[z] Average based on 28 trees.

[y] Random sample of 16 fruit.

[x] Random sample of 50 fruit.

[w] 1 = nil or sparsely scattered; 2 = bloom over half of canopy; 3 = canopy fully covered with bloom.

[v] 1 = nil or few scattered shoots; 2 = half-covered with new growth; 3 = new growth over entire canopy.

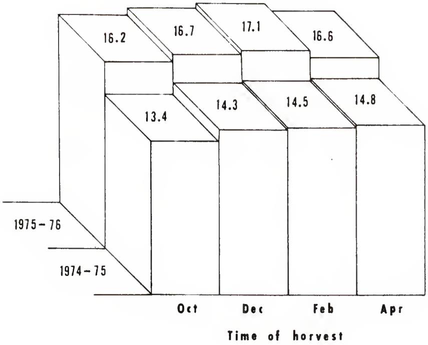
[u] Means in each column followed by the same letter do not differ statistically at the 0.01 level.

[t] 38.6 kg standard box.

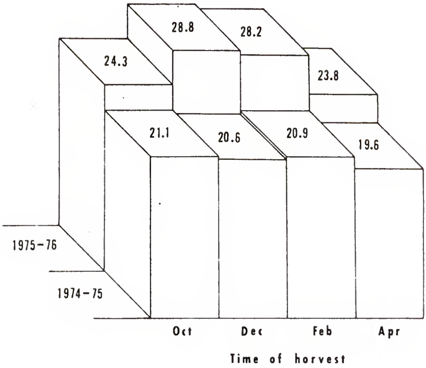
[s] $\text{Solids/tree} = (\text{boxes} \times \text{wt}) \times \%\ \text{juice} \times \frac{\%\ \text{TSS}}{100}$

Fig. 4. Effect of date of harvest on yield in boxes and kg solids per tree of 'Marsh' grapefruit.

Yield Boxes/tree



Yield kg Solids / tree



dates of harvest were initially applied during an "off" year. This would tend to reduce the effects of a late harvest on subsequent yields, as was previously reported (20, 34). Moreover, fruit were harvested in Dec. of the second year in the previous work, instead of leaving fruit on the tree until late in both seasons as was done in this experiment. Thus, fruit continued to increase in size, which compensated at least in part for the depressing effect of late harvest the previous year. Holding 'Marsh' fruit late did not have as pronounced a depressing effect on yield the following year as it did with 'Valencia'; neither did fruit drop play the role of reducing yields of 'Marsh' as it did with 'Hamlin'. However, previous work has shown that holding fruit on the tree later (May) than in this experiment did result in considerable fruit drop (2, 22). Yields as kg solids were calculated even though they have no application because grapefruit are not generally sold on this basis.

The pattern of yields was changed when yields were measured as kg solids in that yields decreased, as the season progressed the first year, due to decreases in both TSS and percent juice. Kilogram solids per tree increased to a maximum during Dec. and Feb. the second year, the yield for the latest harvest date, Apr., being

about the same as or slightly less than for the first harvest in Oct. These yields were, of course, reflections of the volume yields, TSS and percent juice, with the lower TSS and percent juice causing kg solids to drop more sharply for the Apr. harvest than did yields on a volume basis. It is more important to consider the influence of date of harvest on attainment of first legal maturity in the fall, since grapefruit generally demands a higher price early in the season. This cannot be obtained from these data; however, previous work has indicated that date of harvest did not have a pronounced effect on juice quality the following year (73).

Carbohydrates

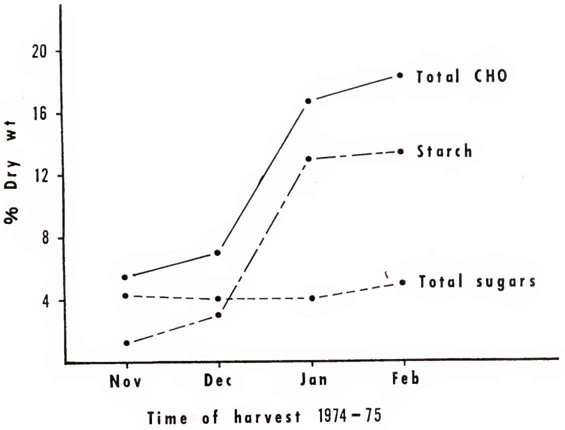
Levels of CHO's were studied in two ways in order to determine whether they were related to date of harvest and the influence of date of harvest on yield. Samples of leaves were analyzed from the trees harvested at each respective date, and leaves from all trees were analyzed at certain times during the fruiting cycle. Seasonal changes in levels of total CHO's and the various fractions of total CHO's of the 3 cultivars cannot be compared, however, because they were sampled at different times of the year due to differing seasons of maturity for each cultivar.

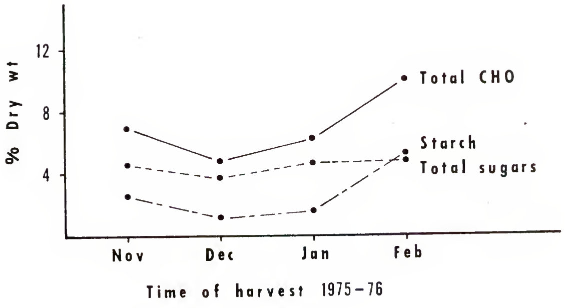
'Hamlin' Orange

There are no values in the literature with which CHO data for 'Hamlin' can be compared. In general, total CHO's followed the patterns reported for 'Valencia' (6, 35, 49, 79) and 'Marsh' in that they rose rapidly from Dec. to Feb. (Fig. 5). Relative amounts of starch and total reducing sugars did not, however, follow these patterns. Levels of starch in 1974-75 were much higher than those reported for 'Valencia', and sugars were higher most of the 1975-76 season but were equalled by starch in Feb. Total CHO's and starch were appreciably lower the second year, while sugars were about the same. Thus, the primary difference was in the high starch level in 1974-75.

The reason for this is not known. One would not expect CHO levels found at various dates of harvest the first season to influence those of the second year because they were from leaves produced in different years. It is noteworthy that 1975-76 yields were lower although the previous season had the highest level of total CHO's. Yield from these trees was not obtained in 1976-77, but records of the grower indicated that the entire block averaged 14.5 boxes per tree. Thus, CHO levels do not appear to be related to yield.

Fig. 5. Changes in carbohydrate content of 'Hamlin' orange leaves during 2 harvest seasons.


20
16
12
8
4
% Dry wt
Total CHO
Starch
Total sugars
Nov
Dec
Jan
Feb
Time of harvest 1974-75


12
8
4
% Dry wt
Total CHO
Starch
Total sugars
Nov
Dec
Jan
Feb
Time of harvest 1975-76

Total soluble solids were considerably higher the second season than the first season, and the kg solids per tree were also higher the first year. The reason for high juice CHO's in a year of low leaf CHO's and low juice CHO's in a year of high leaf CHO's is not apparent. There were no climatic differences that appear related. Precipitation from May to Oct. 1974 and May to Oct. 1975, respectively, differed by only 20 mm (Appendix Table D).

There were no differences in mean levels of total CHO, sugars or starch among trees corresponding to each date of harvest treatment when samples of leaves were taken in Nov. just prior to the first harvest (Table 5). Total CHO's in Feb. 1976 were lower in leaves of trees harvested in Feb., as a result of lower starch, than those from the Nov., Dec. or Jan. plots, which did not differ. One might draw the conclusion that reduction in yields the second year, which was associated with the Feb. harvest of the first year, might have resulted from lower leaf CHO's for the Feb. plots just before the 1975 bloom. There were, however, no indications of reduced bloom or vegetative growth (Table 2). Admittedly, evaluations of bloom and growth were visual ratings, but there were no obvious differences and bloom was sufficiently heavy to preclude a slight reduction in

Table 5. The influence of date of harvest on carbohydrate content of 'Hamlin' orange leaves.[z]

Date of harvest	Time of leaf sampling (% dry wt)									
	Nov. 1974, prior to fruit harvest[y]					Feb. 1976, prior to bloom[x]				
	Sugars				Total	Sugars				Total
	Red[v]	Suc[v]	Total	Starch	CHO	Red	Suc	Total	Starch	CHO
Apr.	1.0a	3.3a	4.3a	1.2a	5.5a	2.4a	2.5a	4.9a	7.3a	12.2a
May	0.9a	3.5a	4.4a	1.2a	5.6a	2.7a	2.2a	4.9a	8.8a	13.7a
June	0.8a	3.4a	4.2a	1.3a	5.5a	2.6a	2.2a	4.8a	7.8b	12.6a
July	1.0a	3.6a	4.6a	1.3a	5.9a	2.7a	2.1a	4.8a	5.3c	10.1b
	Nov. 1975, prior to fruit harvest[x]					May 1976, following bloom[w]				
Apr.	1.8a	2.6a	4.3a	2.6a	6.9a	3.4a	4.2a	7.6a	10.6a	18.2a
May	1.9a	2.7a	4.6a	2.4a	6.9a	3.5a	3.2a	6.7a	11.0a	17.0a
June	1.7a	2.4a	4.1a	2.6a	6.7a	3.5a	3.5a	7.0a	11.5a	16.5a
July	1.8a	2.5a	4.3a	2.5a	6.7a	3.3a	4.2a	7.5a	10.6a	18.1a

[z]Means in each column followed by the same letter do not differ statistically at the 0.01 level.

[y]Mature leaves from spring flush 1974.

[x]Mature leaves from spring flush 1975.

[w]Young leaves from spring flush 1976.

[v]Red = reducing; Suc = sucrose.

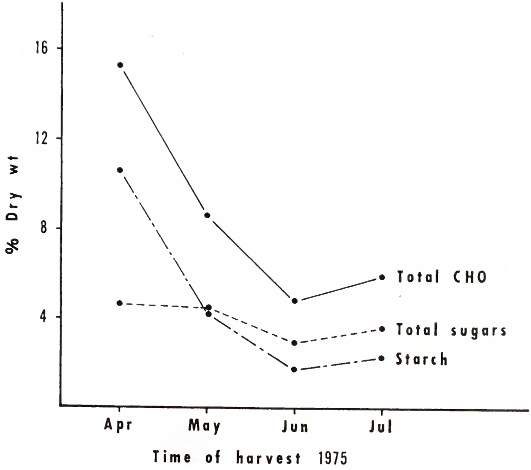
bloom having been responsible for the difference. Leaves from the 1976 spring flush sampled in May from all trees showed no differences among plots in CHO levels (Table 5).

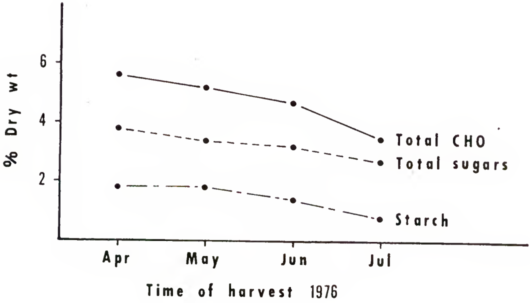
'Valencia' Orange

Much research has been conducted on the relationship of leaf CHO levels of 'Valencia' orange to the flowering and fruiting of citrus but all under conditions climatically quite different from Florida. Nevertheless, changes in CHO levels in 'Valencia' leaves in this experiment are in general agreement with other reports in that total CHO, sugars and starch decreased during the spring, reaching a minimum in the summer months (Fig. 6). The pattern for the second year also showed a decrease, but levels were much higher in 1975 than in 1976, particularly in Apr. Thus the seasonal decline in CHO's was sharper the first season than the second. It could be concluded that higher levels of CHO in 1975 were due to the lighter crop load, the crop of that year being about a third less than in 1976, as has been reported from other areas (35, 47, 50, 51).

Total soluble solids was about the same each year, but the total crop contained more kg solids the second than the first year. Thus, there appears to be no

Fig. 6. Changes in carbohydrate content of 'Valencia' orange leaves during 2 harvest seasons.


16
12
8
4
% Dry wt
Total CHO
Total sugars
Starch
Apr
May
Jun
Jul
Time of harvest 1975


6
4
2
% Dry wt
Total CHO
Total sugars
Starch
Apr
May
Jun
Jul
Time of harvest 1976

relation between high CHO's in the leaves and yields as measured as kg solids.

Levels of leaf CHO's just prior to bloom in 1976 were related to fruit harvest date in the spring and summer of 1975 in that the total CHO, sugars and starch were all generally reduced by later harvest dates (Table 6).

There was no difference in the amount of the following bloom or vegetative growth despite lower levels of CHO's just before bloom in leaves of plots harvested late (Table 3). Thus, depression of yields for the late-harvested plots must have been due to other reasons. Leaves from new growth of 1976 were sampled in July 1976 to see whether harvest date had any effect on their CHO's, but there was none (Table 6). This suggests that CHO levels in a given spring flush are not affected by the previous season's crop. There were no mean differences in leaf CHO's sampled from all treatments just before harvest in Apr. 1976, indicating that any influence due to late harvest had been overcome by that time (Table 6).

'Marsh' Grapefruit

'Marsh' grapefruit, like 'Hamlin' orange, attains legal maturity in early fall, but unlike 'Hamlin' it

Table 6. The influence of date of harvest on carbohydrate content of 'Valencia' orange leaves.[z]

Date of harvest	Time of leaf sampling (% dry wt)									
	Apr. 1975, prior to fruit harvest[y]					Feb. 1976, prior to bloom[x]				
	Sugars				Total	Sugars				Total
	Red[v]	Suc[v]	Total	Starch	CHO	Red	Suc	Total	Starch	CHO
Apr.	0.9a	3.7a	4.6a	10.4a	15.0a	2.1a	2.7a	4.8a	2.6ab	7.4a
May	0.8a	3.6a	4.4a	10.4a	14.8a	2.3a	2.3b	4.6b	2.7a	7.3a
June	0.7a	3.7a	4.4a	10.3a	14.7a	2.2a	2.4b	4.6b	2.5b	7.1ab
July	0.8a	3.6a	4.4a	10.3a	14.7a	2.2a	2.4b	4.6b	2.3b	6.9a
	Apr. 1976, prior to fruit harvest[x]					July 1976, following bloom[w]				
Apr.	1.8a	2.0a	3.8a	1.8a	5.6a	2.0a	1.1a	3.1a	0.6a	3.7a
May	1.8a	1.9a	3.7a	1.8a	5.5a	2.1a	1.0a	3.1a	0.6a	3.7a
June	1.8a	2.0a	3.8a	1.9a	5.7a	1.9a	1.0a	2.9a	0.7a	3.6a
July	1.8a	1.8a	3.6a	1.8a	5.4a	2.1a	1.0a	3.1a	0.7a	3.8a

[z]Means in each column followed by the same letter do not differ statistically at the 0.01 level.

[y]Mature leaves from spring flush 1974.

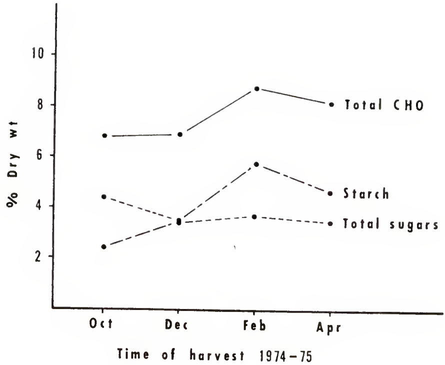
[x]Mature leaves from spring flush 1975.

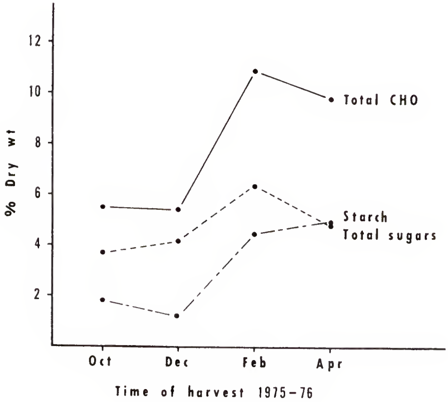
[w]Young leaves from spring flush 1976.

[v]Red = reducing; Suc = sucrose.

can be held on the tree almost as late as 'Valencia'. Thus, leaves were analyzed for CHO's from Oct. through Apr., the common harvest period for grapefruit. In general, total CHO, starch and sugars of 'Marsh' leaves increased until just before bloom, Feb., and decreased after bloom, Apr., in 1974-75. The same trend was true in 1975-76 except that starch continued to increase until Apr. Starch levels were higher than those of total sugars in 1974-75, but starch levels were lower than sugars in 1975-76, except for Apr., when they were about equal (Fig. 7). These data agree with results from Arizona in that maximum CHO levels were reached in Feb., before the bloom. Accumulation of CHO's started earlier in Florida, however. The reason starch was the dominant CHO fraction the first year while sugars dominated the second year is not known. Starch should accumulate under cooler conditions, but temperatures in winter months of 1975-76 were slightly cooler than in 1974-75, the year in which starch exceeded sugars. Total CHO's were higher during much of 1975-76, the year of the heaviest crop load. Thus, crop load does not appear to have affected CHO levels. It was not possible to determine the influence of all of the different harvest dates on levels of leaf CHO's just

Fig. 7. Changes in carbohydrate content of 'Marsh' grapefruit leaves during 2 harvest seasons.


10
8
6
4
2
% Dry wt
Total CHO
Starch
Total sugars
Oct
Dec
Feb
Apr
Time of harvest 1974-75


12
10
8
6
4
2
% Dry wt
Total CHO
Starch
Total sugars
Oct
Dec
Feb
Apr
Time of harvest 1975-76

prior to the next bloom, because the last harvest date occurred after the bloom period. It could be concluded that leaves harvested in Feb. 1976, just before bloom, reflected the influence of each harvest date for 1974-75 and the influence of the Oct., Dec. and Feb. harvest dates for 1975. This would not be correct, however, because trees of all cultivars had equal CHO levels just before harvest each year, and it has been shown that the fruiting cycle can be reversed in one year by reversing the early and late harvest dates (34, 47). Thus, the date for the Feb. and Apr. harvest plots are actually both Feb. dates. Data from both of these plots were very similar (Table 7), and leaf CHO levels for these harvest dates, Feb. and Apr., were much lower than for the 2 previous harvest dates, indicating a depressing effect of a later date of harvest. The pattern was not completely clear, however, because CHO levels from Oct. plots were still considerably higher than those for Feb. There was no difference in any of the CHO fractions in Oct. 1975 at the initiation of the second season, as with the other cultivars, nor in May 1976 with young leaves of the 1976 spring flush (Table 7). There was no apparent relation between CHO levels before bloom and the amount of bloom and new growth (Table 4).

Table 7. The influence of date of harvest on carbohydrate content of 'Marsh' grapefruit leaves.[z]

Date of harvest	Time of leaf sampling (% dry wt)									
	Oct. 1974, prior to fruit harvest[y]					Feb. 1976, prior to bloom[x]				
	Sugars				Total	Sugars				Total
	Red[v]	Suc[v]	Total	Starch	CHO	Red	Suc	Total	Starch	CHO
Oct.	0.7a	3.7a	4.4a	2.4a	6.8a	3.4a	2.9a	6.3a	5.6b	11.9b
Dec.	0.6a	3.7a	4.3a	2.7a	7.0a	3.1b	3.1a	6.2a	6.9a	13.1a
Feb.	0.8a	3.7a	4.5a	2.4a	6.9a	2.5a	2.9a	6.4a	4.5c	10.9c
Apr.	0.7a	3.9a	4.6a	2.7a	7.3a	3.0b	3.2a	6.2a	4.5c	10.7c
	Oct. 1975, prior to fruit harvest[x]					May 1976, following bloom[w]				
Oct.	1.5a	2.2a	3.7a	1.8a	5.5a	4.0a	1.3a	5.3a	10.5a	15.8a
Dec.	1.6a	2.1a	3.7a	1.9a	5.6a	4.1a	1.1a	5.2a	10.2a	15.4a
Feb.	1.5a	2.2a	3.7a	1.7a	5.4a	4.1a	1.2a	5.3a	9.9a	15.2a
Apr.	1.6a	2.1a	3.7a	1.8a	5.5a	4.4a	1.3a	5.7a	11.5a	17.2a

[z]Means in each column followed by the same letter do not differ statistically at the 0.01 level.

[y]Mature leaves from spring flush 1974.

[x]Mature leaves from spring flush 1975.

[w]Young leaves from spring flush 1976.

[v]Red = reducing; Suc = sucrose.

Relation of CHO's to Date of Harvest and Fruiting

Depression of CHO's by late dates of harvest just prior to bloom in the second season was rather consistent for all cultivars. The lack of any indication that differential CHO levels influenced bloom and vegetative growth was not sufficiently precise to measure differences which might have been noted if quantitative data from tagged shoots had been obtained. It is doubtful, on the other hand, that relating small differences in bloom to yield would be justified. Flowering in citrus is recognized as being excessive in that a very large reduction in flowers is needed to cause a reduction in fruiting. Bloom on these trees appeared normal. Moreover, appearance of the growth flush was good in relation to that which is common to citrus. It is also possible that root growth, which was not measured, could have been influenced, because root growth has been greatly affected by heavy crop loads on mandarin trees, despite the fact that CHO levels in the leaves were similar (48, 83). Such reduced root growth generally results in lack of flowering and dieback. It is possible that CHO levels in other plant organs might be more closely related to yield than those of the leaves; however, applying the data of Moss et al.

(64) to our results, the range of accumulation of total CHO per mature leaf during the harvest season in the 3 cultivars was 3 to 20 times the calculated net photosynthate produced per day.

Future work would be more productive if fewer trees were used and if very precise measurements and a more complete accounting were taken of CHO's in all parts of the tree, including roots. Carbohydrates may not be the limiting factor, of course. If this is true, determination of endogenous growth regulators, as has been suggested by some, might well reveal them to be the controlling agency in CHO levels in various parts of the tree.

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APPENDIX

Table A. Seasonal changes in CHO in 'Hamlin' orange leaves.[z]

Date of harvest	Sugars: Red.[y]	Suc[y]	Total	Starch	Total CHO
			(% dry wt)		
			1974-75		
Nov.	1.0ab	3.3b	4.3b	1.2d	5.5c
Dec.	0.7c	3.2b	3.9c	3.1c	7.0c
Jan.	9.0bc	2.9c	3.8c	13.0b	16.7b
Feb.	1.2a	3.7a	4.9a	13.4a	18.3a
			1975-76		
Nov.	1.8b	2.6a	4.4b	2.6b	7.0b
Dec.	1.9b	1.8b	3.7c	1.2c	4.9d
Jan.	2.7a	2.0b	4.7ab	1.6c	6.3c
Feb.	2.7a	2.1b	4.8a	5.3a	10.1a

[z]Means in each column followed by the same letter do not differ statistically at the 0.01 level.

[y]Red = reducing; Suc = sucrose.

Table B. Seasonal changes in CHO in 'Valencia' orange leaves.[z]

Date of harvest	Sugars: Red [y]	Sugars: Suc [y]	Sugars: Total	Starch	Total CHO
			(% dry wt)		
			1975		
Apr.	0.9b	3.7a	4.6a	10.4a	15.0a
May	0.7b	3.6a	4.3a	4.2b	8.5b
June	0.3c	2.6b	2.9c	1.8c	4.7d
July	2.1a	1.5c	3.6b	2.3c	5.9c
			1976		
Apr.	1.8a	2.0a	3.8a	1.8a	5.6a
May	2.1a	1.3b	3.4ab	1.8a	5.2a
June	2.1a	1.1bc	3.2b	1.4b	4.6b
July	1.9a	0.9c	2.7c	0.8c	3.5c

[z]Means in each column followed by the same letter do not differ statistically at the 0.01 level.

[y]Red = reducing; Suc = sucrose.

Table C. Seasonal changes in CHO in 'Marsh' grapefruit leaves.[z]

Date of harvest	Sugars Red[y]	Suc[y]	Total	Starch	Total CHO
			(% dry wt)		
			1974-75		
Oct.	0.7b	3.7a	4.4a	2.4b	6.8b
Dec.	0.6b	2.9b	3.5b	3.4b	6.9b
Feb.	0.6b	3.1b	3.7b	5.1a	8.8a
Apr.	0.9a	2.6c	3.5b	4.8a	8.3a
			1975-76		
Oct.	1.5b	2.2b	3.7c	1.8c	5.5b
Dec.	2.2b	2.0bc	4.2bc	1.2d	5.4b
Feb.	3.5a	2.9a	6.4a	4.5b	10.9a
Apr.	3.3a	1.5c	4.8b	4.9a	9.7a

[z]Means in each column followed by the same letter do not differ statistically at the 0.01 level.

[y]Red = reducing; Suc = sucrose.

Table D. Monthly air temperature (average) and precipitation at Clermont, Florida.[z]

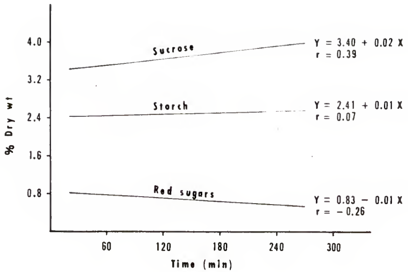
Month	Temp (°C)			Prec (mm)		
	1974	1975	1976	1974	1975	1976
Jan.	20.8	18.2	13.3	12	66	15
Feb.	16.6	19.3	17.2	31	60	6
Mar.	21.6	20.4	21.6	99	21	28
Apr.	21.9	23.2	21.7	22	68	81
May	25.3	24.8	24.4	121	182	127
June	25.2	27.2	25.9	307	149	348
July	26.8	26.9	27.5	198	184	248
Aug.	27.6	27.7	27.5	121	214	248
Sep.	27.7	26.8	26.4	184	174	125
Oct.	22.3	24.7	21.7	6	54	43
Nov.	19.2	19.3	16.8	6	68	87
Dec.	15.6	15.2	15.3	46	27	65
Total				1153	1267	1421

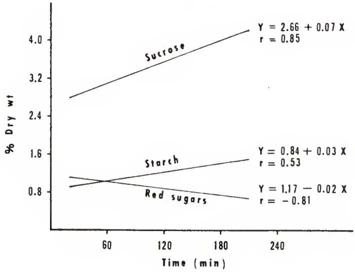
[z]N.O.A.A. 1974, 1975, 1976. Climatological data. Florida. *Environ. Data Serv.* 78:1-12; 79:1-12; 80:1-12.

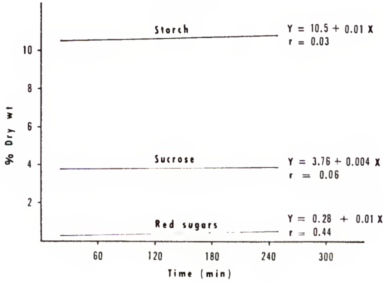
Fig. A. Daily changes in carbohydrates of 'Marsh' grapefruit leaves, Nov. 1974. Zero time 9:30 AM.

Fig. B. Daily changes in carbohydrates of 'Hamlin' orange leaves, Apr. 1975. Zero time 9:30 AM.

Fig. C. Daily changes in carbohydrates of 'Valencia' orange leaves, Oct. 1974. Zero time 9:30 AM.


Sucrose
Y = 3.40 + 0.02 X
r = 0.39
Starch
Y = 2.41 + 0.01 X
r = 0.07
Red sugars
Y = 0.83 − 0.01 X
r = − 0.26
% Dry wt
4.0
3.2
2.4
1.6
0.8
60
120
180
240
300
Time (min)


Y = 2.66 + 0.07 X
r = 0.85
Sucrose
Starch
Y = 0.84 + 0.03 X
r = 0.53
Red sugars
Y = 1.17 − 0.02 X
r = − 0.81
% Dry wt
4.0
3.2
2.4
1.6
0.8
60
120
180
240
Time (min)


Starch
Y = 10.5 + 0.01 X
r = 0.03
Sucrose
Y = 3.76 + 0.004 X
r = 0.06
Red sugars
Y = 0.28 + 0.01 X
r = 0.44
% Dry wt
10
8
6
4
2
60
120
180
240
300
Time (min)

BIOGRAPHICAL SKETCH

Juan Manuel Ramirez was born October 17, 1946, at Guadalajara, Jalisco, Mexico. He attended elementary school at Autlan, Jalisco, and graduated from high school in June 1960. He studied in the National School of Agriculture, Chapingo, from February 1962 until February 1969, when he obtained the degree of Ingeniero Agronomo Especialista en Fitotecnia.

He was a research scientist from March 1969 until February 1973 in the Departamento de Fruticultura del Instituto Nacional de Investigaciones Agricolas.

He entered the Graduate School of the University of Florida in March 1973, where he was graduate assistant in the Department of Fruit Crops from March 1973 until June 1976. He received the degrees Master of Science (Horticultural Science) in March 1975 and Doctor of Philosophy (Horticultural Science) in June 1977.

He is married to the former Maria Elena Guevara and is father of a daughter, Erika. He is a member of the Florida State Horticultural Society and Gamma Sigma Delta.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Alfred H. Krezdorn

Alfred H. Krezdorn, Chairman
Professor of Fruit Crops

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

James Soule

James Soule
Professor of Fruit Crops

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Thomas E. Humphreys

Thomas E. Humphreys
Professor of Botany

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Robert H. Biggs

Robert H. Biggs
Professor of Fruit Crops

This dissertation was submitted to the Graduate Faculty of the College of Agriculture and to the Graduate Council, and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

June, 1977

Jack L. Fry

Dean, College of Agriculture

Dean, Graduate School